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PRINCIPAL INVESTIGATOR: James Dean, M.D., Ph.D.

CONTRACTING ORGANIZATION: Fred Hutchinson Cancer Research Center
Seattle, WA 98109

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James Dean, M.D., Ph.D.

Email: jdean@fhcrc.org

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14. ABSTRACT
The Purpose of this proposal is to examine how senescence in the prostate may be caused bymedical treatments for prostate cancer, and to identify senescence-associated factors whichmay mediate resistance of neoplastic epithelium. The effects of standard and targetedtherapeutics on senescence-mediated resistance will be determined. To date, our majorfindings present a mixed picture of chemotherapy-induced senescence. Senescence-associatedβ-galactosidase staining has not identified significant chemotherapy-induced senescence, butquantitation of gene expression changes reveal a pervasive pattern of senescence changes.Assays for additional markers of senescence and senescence-associated factors have beenoptimized and validated on a Tissue Microarray of Aging and Prostate Cancer. Correlation ofchronological aging and senescence is seen. These assays will be used next on chemotherapy-treated tissues. Finally, our clinical trial of neoadjuvant anti-IGF-1R antibody therapywith combined androgen deprivation prior to prostatectomy will examine the clinical effectsof abrogating a pathway which is altered in senescence.

15. SUBJECT TERMS
prostate, cancer, senescence, paracrine, resistance, chemotherapy, androgen deprivation

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Introduction

Deaths due to prostate cancer- the second leading cause of cancer death in men in the United States - could be prevented with more effective treatments. Overcoming tumor cell resistance to the effects of androgen deprivation and chemotherapies would significantly improve the morbidity and mortality of prostate cancer. We ***hypothesize*** that the induction of cellular senescence in the tumor microenvironment by androgen deprivation and cytotoxic chemotherapy promotes the resistance and survival of carcinoma cells. We further ***hypothesize*** that targeting senescence-associated pro-survival paracrine factors will enhance the effects of these therapies and enhance response rates.

To address these hypotheses, we have three aims: First, to identify senescence changes in prostate tissue induced by androgen deprivation and chemotherapy, specifically focusing on identifying factors with the potential to influence the survival/resistance of neoplastic epithelium via paracrine mechanisms. Second, to evaluate the effects of inhibiting specific senescence-associated pro-survival factors using *in vivo* models. Third, to develop and execute clinical trials designed to inhibit senescence-associated paracrine survival mechanisms and determine if enhanced tumor responses can be achieved.

Body

The following summarizes the research accomplishments of the first year of this proposal, as associated with each task in the Statement of Work.

Technical Objective 1: To determine the effects of chemotherapy and androgen ablation therapy on the frequency, type, and location of senescent cells in the prostate, and to identify changes in levels of senescenc-associated signaling molecules found in those tissues.

Objective 1a. *Identify senescent cells in pre- and post-chemotherapy prostate tissues from 58 patients.*

Task 1. Perform histochemical staining of a defined set of senescence biomarkers. (Months 1-6) We have completed histochemical staining for Senescence-Associated β-Galactosidase (SA-β-Gal) on post-chemotherapy prostate tissues from a random sample consisting of 25 of the treated patients. Unfortunately, despite an assay optimized for maximal sensitivity and histochemical clarity, only 6 of the patients had detectable staining. Of the tissues demonstrating staining, intensity was highly variable (one intense, five weak), and the localization of the staining demonstrated irregular clustering (from zero to 20 glands per low power field within the same tissue section), rather than widespread distribution of staining. In consultation with our collaborating pathologist, Dr. Larry True, our judgement is that staining of the pre-treatment needle core biopsy tissues is unlikely to demonstrate statistically significant increases in staining after chemotherapy treatment.

We have also established staining conditions for the immunohistochemical staining of two well-established markers of senescence, p16(INK4a) and DcR2. To establish these conditions, antigen retrieval, blocking, titration of primary antibodies, and detection methods were optimized. These conditions have been validated using a Tissue Microarray of Aging and Prostate

Cancer to avoid squandering the limited tissue available from the chemotherapy clinical trial. We developed this unique tissue microarray resource specifically for the purpose of examining the correlations of senescent cells and senescence-associated factors with aging in benign and neoplastic prostate tissues. In this tissue microarray, prostate tissue cores containing benign and neoplastic epithelium were collected from the prostatectomy blocks of 25 men aged less than 50 and 25 men aged greater than 70, in quadruplicate. Staining of chemotherapy-treated tissues using these optimized protocols is ongoing.

Task 2. Analyze stained tissues, quantitating frequency of positive staining cells in the epithelium versus stroma, in or near benign versus neoplastic prostate glands. (Months 2-8) Analysis has been completed for the tissue microarray of aging as described above. We have quantitated the intensity and the frequency of staining in the benign epithelium, neoplastic epithelium, and the stroma, using a 0-2 point scale for staining intensity.

Task 3. Perform statistical analyses to determine the significance of staining differences between the various compartments and differences before and after chemotherapy. (Months 2-8) Per our prediction, we find that both p16 and DcR2 both have statistically significant increases in staining of benign epithelium and stromal compartments in aged men, when compared to young men (**FIGURE 1**). The magnitude of this increased staining ranges from 2.3 to 3.7-fold more staining in aged men, compared to young men. There are also highly significant increases in the staining scores of the neoplastic epithelium compared to the benign epithelium, irrespective of patient age for both of these markers. Thus, we have validated these staining protocols and these protein biomarkers as tools for the investigation of senescence in prostate tissue and are prepared to apply these protocols to the chemotherapy-treated tissues. We have also provided evidence to support the long-standing hypothesis that chronological aging is correlated with increased senescence, specifically in the prostate.

Objective 1b. *Identify senescent cells in pre- and post-androgen ablation prostate tissues from 48 patients.*

Task 4. Perform histochemical staining of a defined set of senescence biomarkers. (Months 12-20) - pending To date, 31 patients have been enrolled, and 28 have been treated on this protocol of neoadjuvant androgen ablation therapy given prior to prostatectomy. Given our results as described above in Task 1, we will proceed with staining a subset of the treated patients to determine whether Senescence-Associated β-Galactosidase staining will yield interpretable results when compared to pre-treatment tissue staining.

Task 5. Analyze stained tissues, quantitating frequency of positive staining cells in the epithelium versus stroma, in or near benign versus neoplastic prostate glands. (Months 13-22)-pending

Task 6. Perform statistical analyses to determine the significance of staining differences between the various compartments and differences before and after chemotherapy. (Months 13-22)-pending

Objective 1c. *Examine senescence-associated signaling molecules in pre- and post-chemotherapy prostate tissues from 58 patients.*

Task 7. Perform histochemical staining of secreted signaling molecules. (Months 3-18) As with the p16 and DcR2 immunohistochemistry described above, we have also employed the tissue microarray of aging and prostate cancer to optimize the staining for a set of senescence-associated, secreted signaling molecules. Based on the proteomics data as discussed in the Pre-

liminary Data of our grant and further work performed to complete our manuscript in preparation, *Quantitative proteomic analysis of proteins released from prostate stromal cells with activation of the program of cellular senescence*, we chose to investigate the staining of STC1, GDF15, CXCL1, IL8 and IGF-1R first. Staining has been optimized and completed on the tissue microarray of aging for STC1, GDF15, CXCL1 and IL8. IGF-1R staining is ongoing.

Task 8. Analyze stained tissues, quantitating staining intensities and locations of positive staining; epithelium versus stroma, in or near benign versus neoplastic prostate glands. (Months 4-20) STC1 and GDF15 staining patterns have been analyzed. We quantitated the intensity and the frequency of staining in the benign epithelium, neoplastic epithelium, and the stroma, using a 0-2 point scale for staining intensity as with p16 and DcR2 analysis.

Task 9. Perform statistical analyses to determine the significance of staining differences between the various compartments and differences before and after chemotherapy. (Months 4-20) Analyses to date have failed to demonstrate an age-dependent change in the staining intensities, frequencies, or patterns for the GDF15 and STC1 proteins. For GDF15, there is a statistically significant increase in the staining of neoplastic epithelium, compared to benign epithelium, for both young and old men. In the case of STC1, only the young men have a statistically significant increase in the staining of neoplastic epithelium. No significant stromal GDF15 staining was visualized. By contrast, the increased STC1 staining seen in the stroma bordered on the statistically significant ($p=0.09$). In fact, STC1 is the first protein we have examined with intense and widespread stromal staining patterns. Preliminary examination of the CXCL1 staining patterns finds sparse staining of scattered stromal cells, and no epithelial staining. Analyses continue.

Objective 1d. *Examine senescence-associated signaling molecules in pre- and post-androgen ablation prostate tissues from 48 patients.*

Task 10. Perform histochemical staining of secreted signaling molecules. (Months 12-36)-pending, see above.

Task 11. Analyze stained tissues, quantitating staining intensities and locations of positive staining; epithelium versus stroma, in or near benign versus neoplastic prostate glands. (Months 13-36)-pending

Task 12. Perform statistical analyses to determine the significance of staining differences between the various compartments and differences before and after chemotherapy. (Months 13-36)-pending

Objective 1e. *Correlate observed senescence staining results from Objectives 1a-1d with clinical outcomes and gene expression data.*

Task 13. Collate senescence staining results and clinical outcomes. (Months 4-40) - pending Attempts to identify a secreted senescence biomarker with altered levels due to therapeutic treatments are ongoing and critical to allow these correlative studies.

Task 14. Collaborate with Nelson laboratory members performing the parallel expression studies to correlate staining patterns with gene expression changes. (Months 4-40) - pending In a complementary approach to the histochemical and immunohistochemical studies described above, we have performed quantitative real-time PCR experiments on RNA isolated from pre- and post-chemotherapy treated tissues. Laser capture microscopy was first used to isolate cells from each of the three tissue compartments of interest: neoplastic epithelium, benign epithelium, and stroma. After amplification, quantitative real-time PCR was used to assay

changes in expression levels of senescence biomarkers, as well as senescence-associated secreted proteins. We can demonstrate statistically significant increases ranging from 3.7- to 28-fold in the expression of the senescence biomarkers p16 and p21 in all three compartments of the prostate, as a result of chemotherapy treatment (**FIGURE 2**). CXCL1 (GROa) and IL8, currently under investigation as described above in Objective 1c, also have statistically significant increases in gene expression (from 2.6- to 22-fold) in all three compartments after chemotherapy treatment. Finally, MMP3 and IGFBP2 are among the senescence-related proteins identified in our mass spectrometry experiments and have increased expression after chemotherapy treatment (1.4- to 36-fold increase). Further work is ongoing and will be used, in part, to guide further immunohistochemistry efforts.

Technical Objective 2. To examine resistance of senescence-induced carcinogenesis and tumor progression to the effects of docetaxel and/or the anti-IGF-IR antibody IMC-A12 in a nude mouse xenoplant model.

Objective 2a. *Obtain regulatory approval for animal xenoplant trials.*

Task 15. Write animal protocol for therapy trials. (Months 1-2). This has been completed.

Task 16. Obtain necessary animal review board approval. (Months 2-5). Fred Hutchinson Cancer Research Center Institutional Animal Care and Use Committee approval has been obtained for the proposed studies (IACUC #1743). USAMRMC Animal Care and Use Review Office (ACURO) approval has also been obtained.

Objective 2b. *Determine the resistance of senescent-dependent carcinogenesis and early tumors to docetaxel chemotherapy.*

Task 17. Culture cells and prepare cellular recombinants with BPH1 or PC3 and primary prostate stromal cells, senescent, or not. Perform sub-kidney capsule implantation surgeries. After 2 weeks of recovery, treat mice with weekly docetaxel for 3 weeks. 2 months after completion of therapy, sacrifice mice and retrieve xenoplanted kidneys. (Months 5-12) - pending
The experimental work proposed in this Objective, as with Objectives 2c and 2d have largely been deferred to allow commitment of time and energies into developing the clinical trial described below in Objective 3a, Task 26. However, we have completed a small series of studies designed to complement these therapy trials. In these studies, we have now demonstrated that replicative senescence, as well as senescence induced by hydrogen peroxide, can mediate the growth stimulatory effects we previously described in our proposal. Secondly, we have now demonstrated that the NPF cell line, in addition to the PSC27 and PSC36 cell lines, is capable of mediating senescence-dependent growth stimulation. The results of these experiments confirm the consistency of prostate stromal senescence-dependent epithelial stimulation.

Interestingly, further examples of carcinogenesis have not been seen to date. This work will serve as useful control data for our therapy trials and also further solidifies the generalizability of this phenomenon. Based on these data, we will focus on the ability of chemotherapy to prevent benign and neoplastic prostate epithelial growth stimulation by senescent prostate stromal cells. We will, however, continue to monitor for evidence of carcinogenesis. With completion of the regulatory applications required to undertake the clinical trial in Objective 3a as discussed below, the experiments outlined in Objectives 2b, 2c and 2d will proceed accordingly.

Task 18. Measure resulting graft sizes, fix and embed the tissues, then stain with Hematoxylin and Eosin and immunohistochemistry. Evaluate for invasiveness, senescent cell populations and neoplastic morphologies. (Months 8-14 - pending)

Task 19. Perform statistical analyses to determine significance of chemotherapy-induced changes in the senescent-stimulated, compared to the pre-senescent recombinants. (Months 10-16) - pending

Objective 2c. *Determine the effect of IMC-A12 on senescent-dependent carcinogenesis and early tumors.*

Task 20. Culture cells and prepare cellular recombinants with BPH1 or PC3 and primary prostate stromal cells, senescent, or not. Perform sub-kidney capsule implantation surgeries. After 2 weeks of recovery, treat mice with thrice weekly IMC-A12 injections continuously for 2 months, then sacrifice mice and dissect out kidneys. (Months 7-12) - pending. For further details please see explanation under Objective 2b, Task 17.

Task 21. Measure resulting graft sizes, fix and embed the tissues, then stain with Hematoxylin and Eosin and immunohistochemistry. Evaluate for invasiveness, senescent cell populations and neoplastic morphologies. (Months 10-14) - pending

Task 22. Perform statistical analyses to determine significance of chemotherapy-induced changes in the senescent-stimulated, compared to the pre-senescent recombinants. (Months 12-18) - pending

Objective 2d. *Determine the senescence-dependent resistance of advanced tumors to docetaxel chemotherapy and its modulation by IMC-A12.*

Task 23. Culture cells and prepare cellular recombinants with BPH1 or PC3 and primary prostate stromal cells, senescent, or not. Perform sub-kidney capsule implantation surgeries. Two months after implantation, treat mice with weekly docetaxel for 3 weeks, with or without IMC-A12 thrice weekly treatments. 3 weeks after completion of therapy, sacrifice mice and dissect out kidneys. (Months 10-18) - pending. For further details please see explanation under Objective 2b, Task 17.

Task 24. Measure resulting graft sizes, fix and embed the tissues, then stain with Hematoxylin and Eosin and immunohistochemistry. Evaluate for invasiveness, senescent cell populations and neoplastic morphologies. (Months 13-20 - pending)

Task 25. Perform statistical analyses to determine significance of chemotherapy-induced changes in the senescent-stimulated, compared to the pre-senescent recombinants. (Months 15-22) - pending

Technical Objective 3: To develop and execute clinical trials evaluating the effectiveness of inhibiting senescence-associated modulators of cancer cell survival in the treatment of prostate cancer. The lead candidate for such targeting is currently IGF1R in the insulin-like growth factor pathway. Other targets identified in Specific Aims/Technical Objectives 1 and 2 may also present opportunities for treatment of more novel targets. Optimal target selection and clinical trial design will be determined during year 2 of the proposal.

Objective 3a: *Participate in the execution and analysis of a Phase II clinical trial inhibiting senescence-associated modulators of cancer cell survival in combination with androgen ablation*

in the neoadjuvant setting prior to radical prostatectomy. The lead class of modulators are anti-IGF1R antibodies under development by Imclone (IMC-A12) or Pfizer (CP-751,871) which inhibit the insulin-like growth factor pathway. Other candidates/pathways will be considered and optimized during the clinical trial design phase of the project in year 2.

Task 26. Design Clinical Trial (Months 15-18) As part of the educational program detailed in my proposal, I attended the 2007 ASCO/AACR Methods in Clinical Cancer Research workshop, July 27-August 3, 2007, during which time I had the opportunity to further refine the proposed clinical trial as detailed above. Subsequent to this, a Food and Drug Administration Investigational New Drug application was submitted and duly approved (IND 79729). The clinical trial has also been approved by the Scientific Review Committee of the Fred Hutchinson/University of Washington Cancer Consortium. The clinical trial is currently being reviewed by the University of Washington Human Subjects Review Committee V (institutional review board). We anticipate submitting the necessary regulatory paperwork to the U.S. Army Medical Research and Materiel Command's Office of Research Protections, Human Research Protection Office, shortly.

In this clinical trial, 28 patients will be treated with the Imclone anti-IGF-IR antibody IMC-A12 every two weeks simultaneously with combined androgen deprivation therapy (goserelin plus bicalutamide). After three months of this treatment, patients will undergo standard prostatectomy. Tissues excess to the needs of clinical pathological diagnosis and staging will be analyzed for several laboratory endpoints, including gene expression changes, and changes in cellular proteins by immunohistochemistry. All patients will have a pre-treatment prostate biopsy for the purposes of comparison with the prostatectomy tissues. The primary endpoint will be a pathological complete response rate predicted to be 20%. This will be compared to historical control data in which 5% of patients treated for 3 months with combined LHRH agonist and testosterone receptor antagonist prior to prostatectomy were found to have pathological complete response. This number of patients is calculated to provide 80% power with 5% one-sided Type I error rate.

Task 27. Develop recruitment materials, hold in-service for clinical providers and study coordinator. (Months 18-24) - pending. As part of our University of Washington Human Subjects Review Committee application, a world-wide web-based recruitment posting has been prepared. Once final approvals for the clinical trial are obtained, this will be posted on the Fred Hutchinson/University of Washington Cancer Consortium, University of Washington Health Sciences and the Fred Hutchinson Cancer Research Center web sites.

Task 28. Recruit, enroll, and treat patients on study. Monitor and report adverse events. (Months 20-55) - pending

Task 29. Analyze pre- and post-treatment tissues for changes senescent cell content and senescence-associated signaling molecule expression as described above (Objectives 1a. and 1c.). (Months 24-46) -

pending

Task 30. Collate and analyze data. (Months 24-50) - pending

Objective 3b*: Design and execute a clinical trial combining docetaxel chemotherapy with an inhibitor of senescence-associated modulation of cancer cell survival in the neoadjuvant setting prior to radical prostatectomy. The anti-IGF1R antibodies such as IMC-A12 (Imclone) or CP-751,871 (Pfizer) are currently the lead candidates for inhibition. Other candidates/pathways will be considered and optimized during the clinical trial design phase of the project in year 3.*

Task 31. Write the protocol and required regulatory documents. (Months 24-30) - pending

Task 32. Obtain regulatory approval from Institutional Review Board. (Months 30-34) - pending

Task 33. Develop recruitment materials, hold in-service for clinical providers and study coordinator. (Months 34-36) - pending

Task 34. Recruit, enroll, and treat patients on study. Monitor and report adverse events. (Months 36-60) - pending

Task 35. Analyze pre- and post-treatment tissues for changes senescent cell content and senescence-associated signaling molecule expression as described above (Objectives 1a. and 1c.). (Months 40-55) - pending

Task 36. Collate and analyze data. (Months 34-60) - pending

Technical Objective 4: To complete data analyses, compile accomplishments and reportable outcomes, and write final project reports and manuscripts.

Objective 4a*: Prepare manuscript 1.*

Task 37. Describe changes in the extent and distribution of senescent cells in the prostate as a function of chemotherapy and androgen ablation therapies. Correlate with alterations in senescence-associated signaling molecules, gene expression studies and clinical outcome measures. (Months 38-44) - pending

Objective 4b: *Prepare manuscript 2.*

Task 38. Describe the effects of docetaxel, mitoxantrone, and IMC-A12 treatment on the nude mouse model of senescence-dependent carcinogenesis and progression. Inter-correlations of presence or absence of tumor, size of grafts, extent and distribution of senescent cells and levels of senescence-associated signaling molecules. (Months 24-30) - pending

Objective 4c*: Prepare manuscript 3.*

Task 39. Describe the clinical efficacy, side effect profile, and laboratory correlates data resulting from the combination of IMC-A12 with androgen ablation. (Months 50-60) - pending

Objective 4d*: Prepare manuscript 4.*

Task 40. Describe the clinical efficacy, side effect profile and laboratory correlate study results from the clinical trail combining IMC-A12 with chemotherapy. (Months 54-60) - pending

Key Research Accomplishments

• We employed the Senescence-Associated-β-Galactosidase histochemical stain to investigate the effects of chemotherapy on induction of senescence in the cells of the prostate. Staining is adequate to identify epithelial cells in which senescence has taken place. Senescence can not be identified in the stromal compartment of the prostate. Despite an optimized assay, it has become clear that senescence changes identified by this method occur too infrequently to allow statistically significant correlations with changes when compared to pre-treatment tissues. Other ap-

proaches to the identification of senescence changes in the prostate as a result of chemotherapy will be required.

• We established the staining conditions for the senescent cell markers p16(INK4a) and DcR2. We have validated these methods using a unique tissue microarray of aging and prostate cancer. For both of these biomarkers of senescence, we have demonstrated statistically significant increases in benign epithelial cell senescence and in stromal senescence that correlate with aging. We have also demonstrated statistically significant increases in neoplastic epithelial staining by these markers when compared to benign epithelial staining, irrespective of patient age.

• We established the staining conditions for the secreted senescence-associated proteins STC1, GDF15, CXCL1, IL8, IGF-1R. Never before examined in the human prostate, we have demonstrated significant association of increased STC1 staining in neoplastic epithelium, compared to benign epithelium. In addition, there is weak (and not statistically significant) association of increased STC1 staining in the stroma that occurs with aging and an even more weak association of decreased STC1 staining that occurs with aging in the benign epithelium. We have also demonstrated a significant increase in GDF15 staining in the neoplastic prostate epithelium, compared to benign epithelium.

• Correlative work has demonstrated treatment-associated gene expression changes in senescence cell markers and senescence-associated proteins. These are consistent with proteomics data we presented at the 2007 AACR Annual Meeting, April 14-18, 2007. There are many chemotherapy treatment-related gene expression changes in the benign epithelium, neoplastic epithelium, and the stromal compartments consistent with senescence changes, as determined by quantitative real-time PCR on samples obtained by laser-capture microscopy.

• Due to the opportunity to move forward the work of Technical Objective 3a, work on Technical Objective 2 has been temporarily delayed. However, work achieved during this interim have further demonstrated that senescent prostate fibroblasts of different patient origin and made senescent by different means, are sufficient to provide statistically significant growth-stimulation to benign prostate epithelial cells.

• We secured essential funding for the clinical trial proposed in Technical Objective 3a. I acquired clinical trials design and implementation training at the ASCO/AACR Methods in Clinical Cancer Research workshop, July 28-August 3, 2007. Significantly ahead of the proposed timeline, we have completed final revisions to the protocol, obtained Investigational New Drug application approval from the Food and Drug Administration and approval of the protocol by the Fred Hutchinson/University of Washington Center Cancer Consortium Scientific Review Committee. Final review by the University of Washington Human Subjects Review Committee V is ongoing. Regulatory paperwork for U.S. Army Medical Research and Materiel Command's Office of Research Protections, Human Research Protection Office review is in preparation and will be submitted shortly.

Reportable Outcomes

Publication:
Dean, JP, Nelson, PS. Profiling influences of senescent and aged fibroblasts on prostate carcinogenesis. Br J Cancer; 2008 Jan 29:98(2):245-249.

Abstract and Poster Presentation:
Dean JP, Coleman I, Martin DB, Nelson, PS. Potential mediators of prostate carcinogenesis identified through quantitative analysis of the extracellular proteome associated with prostate fibroblast senescence [abstract]. In: Proceedings of the 98th Annual Meeting of the American Association for Cancer Research; 2007 Apr 14-18; Los Angeles, CA. Philadelphia (PA): AACR; 2007. Abstract nr 2788.

Manuscript in Preparation:
Dean JP, Coleman I, Martin DB, Nelson, PS. Quantitative proteomic analysis of proteins released from prostate stromal cells with activation of the program of cellular senescence.

Conclusion

The research accomplished to date has demonstrated that senescent cells, as assayed by the Senescence-Associated β-Galactosidase assay, do not appear to accumulate upon treatment with chemotherapy. On the other hand, quantitative real-time PCR quantitation of gene expression changes after treatment with chemotherapy appear to support the accumulation of senescent cells. We have established the immunohistochemical conditions for staining prostate tissues for the senescent cell markers p16 and DcR2 and using a Tissue Microarray of Aging and Prostate Cancer, we have validated these staining protocols and demonstrated a correlation between chronological aging and increased staining of these proteins in benign epithelium and the prostate stroma. We have used a similar approach to establish staining conditions for the senenescence-associated, secreted proteins GDF15, STC1, CXCL1, IL8 and IGF-1R. Results for these proteins have been less encouraging with a failure to demonstrate statistically significant changes in staining with chronological aging. However, examined for the first time in the prostate and in prostate cancer, STC1 does demonstrate the highest levels of stromal staining we've seen of any protein examined to date in these studies. These staining protocols will be essential to ongoing efforts to identify senescence changes that occur with chemotherapy at the protein level. The work accomplished to date will also serve as an excellent control dataset for future comparisons.

Given the successful funding of the clinical trial designed test how abrogation of the senescence-associated insulin-like growth factor signaling pathway can enhance the efficacy of standard combined androgen deprivation, we opted to focus our efforts on the final steps in development of this trial, ahead of the proposed timeline. We have been successful in obtaining Food and Drug Administration Investigational New Drug approval, FH/UW Cancer Consortium Scientific Review Committee approval, and are now in the final stages of obtaining UW Human Subjects Review approval. With the impending submission of our protocol to the U.S. Army Medical Research and Materiel Command's Office of Research Protections, Human Research Protection Office, the regulatory applications process will be at an end and this trial will move forward to

start accruing patients. As a result of this work, we are behind our predicted timeline for the animal trials of the second specific aim. However, the work we have continued to do with this model system has further demonstrated that senescence in prostate stromal cells is a generalizable phenomenon and this work will provide useful control data for the planned therapy trials.

Moving forward, completion of the work detailed for the first Technical Objective will more fully define the relationships of androgen deprivation therapy and chemotherapy with the induction of senescence in the prostate gland. Use of the in vivo mouse model of the effects of stromal senescence on prostate epithelial cells to examine how senescence changes may interfere with therapeutic efficacy. Combined with the proteomics data that has been presented at the 2007 AACR Annual Meeting and for which a manuscript is in preparation, these data may provide the impetus and the insight necessary to allow us to ameliorate a potentially important source of resistance to medical therapies, optimizing outcomes for patients. Finally, the Phase II investigation of the insulin-like growth factor receptor antagonist IMC-A12 together with combined androgen deprivation therapy will start to test whether pathways of importance in senescence changes may also be important in cellular responses to standard medical therapies.

References

None.

Appendices

FIGURE 1. Senescence biomarkers on a Tissue Microarray of Aging and Prostate Cancer

FIGURE 2. Changes in gene expression levels with chemotherapy treatment

FIGURE 1. Senescence Biomarkers on a Tissue Microarry of Aging and Prostate Cancer

A. p16

Tissue Compartment	Staining Score Young Cohort	Staining Score Aged Cohort	2-sample 2-tailed t-test Young vs. Aged
Benign Epithelium	0.95 +/- 2.37	3.21 +/- 8.00	p<0.05
Neoplastic Epithelium	35.9 +/- 39.2	32.7 +/- 32.9	p=0.68
Stroma	0.92 +/- 1.60	2.16 +/- 5.38	p<0.05
2-sample, 2-tailed t-test Benign vs. Cancer	p<0.0001	p<0.0001	

B. DcR2

Tissue Compartment	Staining Score Young Cohort	Staining Score Aged Cohort	2-sample 2-tailed t-test Young vs. Aged
Benign Epithelium	16.5 +/- 22.2	39.1 +/- 32.9	p<0.0001
Neoplastic Epithelium	97.2 +/- 53.7	88.0 +/- 51.1	p=0.43
Stroma	0.19 +/- 0.82	0.70 +/- 2.22	p<0.05
2-sample, 2-tailed t-test Benign vs. Cancer	p<0.0001	p<0.0001	

C. STC1

Tissue Compartment	Staining Score Young Cohort	Staining Score Aged Cohort	2-sample 2-tailed t-test Young vs. Aged
Benign Epithelium	87.8 +/- 57.9	73.8 +/- 46.9	p=0.26
Neoplastic Epithelium	58.3 +/- 46.9	58.0 +/- 46.2	p=0.97
Stroma	57.2 +/- 36.8	67.0 +/- 40.0	p=0.09
2-sample, 2-tailed t-test Benign vs. Cancer	p<0.005	p=0.096	

D. GDF15

Tissue Compartment	Staining Score Young Cohort	Staining Score Aged Cohort	2-sample 2-tailed t-test Young vs. Aged
Benign Epithelium	99.6 +/- 68.1	87.9 +/- 54.2	p=0.23
Neoplastic Epithelium	165.7 +/- 45.0	158.9 +/- 42.3	p=0.48
Stroma	n.s.	n.s.	n.a.
2-sample, 2-tailed t-test Benign vs. Cancer	p<0.0001	p<0.0001	

Legend: Staining for each indicated protein was evaluated by visual inspection of stained tissues. For each tissue core in the Tissue Microarray of Aging and Prostate Cancer, the percentage of cells of a given compartment (Benign Epithelium, Neoplastic Epithelium, or Stroma) at each of three staining levels was estimated. Staining levels were scored as 0= no staining, 1+= light staining, 2+= intense staining. Staining score was obtained by adding the percentage of cells staining 1+ to the product of the percentage of cells staining 2+ times 2. The average and standard deviation of all scored cores was calculated. 2-sample, 2-tailed t-testing was employed to estimate statistical significance of the pairwise differences between the indicated sets.

FIGURE 2. Changes in gene expression levels with chemotherapy treatment

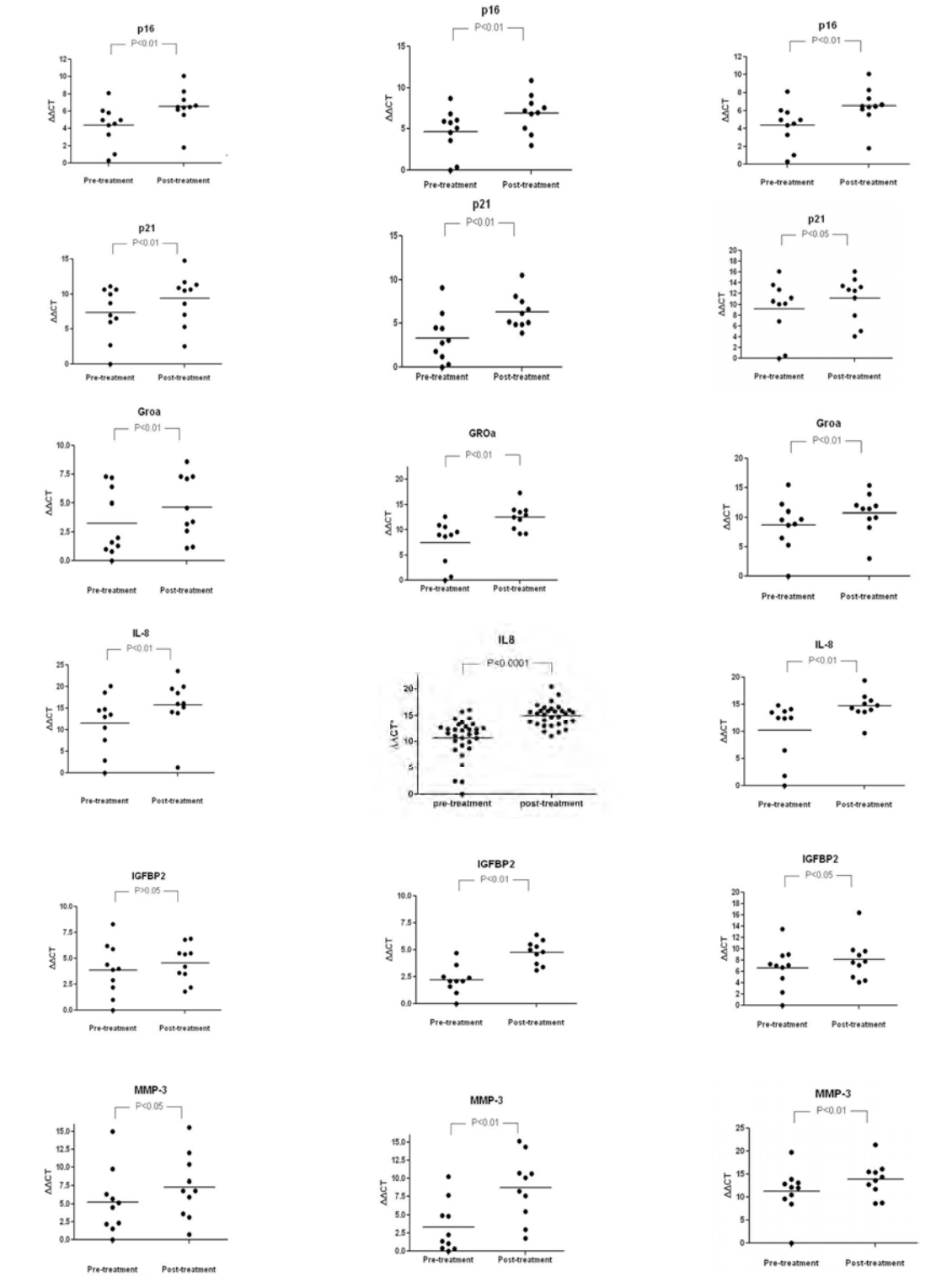

FIGURE 2. Changes in gene expression with chemotherapy treatment. Laser capture microscopy was used to isolate pure populations of the indicated prostate tissue constituents. After amplification and reverse transcription, the presence of the indicated gene transcripts were assayed by quantitative real-time PCR. Each sample was normalized to the house-keeping gene RPL13a, and further normalized to the lowest expressing pre-treatment sample. 2-sample t-tests were used to determine statistical significance of differences between pre-treatment and post-treatment gene expression levels.